The Researching of the Effect of Temperature on Chlorella Growth and Content of Dissolved Oxygen and Content of Chlorophyll

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ABSTRACT: The gradient method was used in this article, for temperature influence in *chlorella* growth and dissolved oxygen content. The result indicated：The *chlorella* is broad to the temperature adaptation scope. *Chlorella* can grow normally in 5~30℃. the optimum temperature is 25℃, and the growth speed of *chlorella*, content of dissolved oxygen and content of chlorophyll reach the maximum.

KEY WORDS: chlorella; growth; dissolved oxygen content; chlorophyll

1 Introduction

There are about 15 types of *chlorella* known by us in the world, and up to more than one hundred types including its mutations. In our country, the common types can be divided into *Chlorella vlgaris, Ch.ellipsoidea* and *Ch.py-renoidosa*. With wide ecological distribution, easy cultivation and fast growth, *chlorella* is a type of excellent material for biologic technology research. Furthermore, it is also a kind of good source for single cell protein (SCP) with rich nutrition. Therefore, *chlorella* has high value of application, and its development and utilization together with spirulina, Dunalielle salina and scenedesmus constitute microalgal biotechnology, which has become a new important branch in the field of biotechnology[1-2]. *Chlorella* is a kind of photoautotroph and able to form a circulation system with a person, that's to say, carbon dioxide (CO_2) exhaled by a person can supply growth of *chlorella*, reversely, Oxygen (O_2) produced by *chlorella* can be used by him, and hence, it possesses important value of exploitation in the field of spaceflight. Simultaneously, as a kind of alkalescent food, *chlorella* contains many kinds of rich and balanced nutritious components like protein, lipid, amylase, meal fiber, Vitamin A, B, C, D and E, microelement, minerals, folic acid, chlorophyll and precious biological activity Vitamin. Besides, *chlorella* contains *chlorella* growth factor, which is the most important component in it and can place premium on interferon, inspire human defense, macrophages in an immunity system, T cell and B cell, as well detoxicate and excrete hazardous substances contaminating environment, such as Dioxins. The influence that temperature and illumination take to marine diatom has been publically reported, and this paper shall make a research in how temperature impacts growth of *chlorella* and change of dissolved oxygen in its solute,

so as to provide basic data for large-scale *chlorella* incubation and comicroalgae's ecological control research.

2 Materials and Methods

2.1 Experimental Materials

Chlorella for experiment: *chlorella pyrenoidosa* from Dalian Aquatic Biological Laboratory Water for experiment: purified water and seawater. The former adopts Hangzhou Wahaha purified water; the latter adopts the sand-leach seawater, which comes from Heishijiao sea area, is used by the Biological Laboratory of Dalian Ocean University, and needs boiling and sterilizing before use.

Medicaments for experiment: $MnSO_4$ solute, KI-NaOH solute and concentrated H_2SO_4. Environment incubator for experiment: adopt domestic HPG-280 illumination incubator, which is adjustable with 5 levels when illumination is less than 14000lx. Equipments for experiment: OPTIZEN2120UV spectrophotometer, ultrasonic processing instrument and centrifuge etc.

2.2 Testing Methods

Use syphon tube to take *chlorella* solute up to a certain concentration to oxygen cylinder so as to be incubated in illumination incubator.

Temperature Settings: put the *chlorella* solute to be used for incubation into incubator, and implement the incubation respectively at 5℃, 10℃, 15℃, 20℃, 25℃, 30℃, 35℃, and measure its concentration, dissolved oxygen and protein content every 2.5 hours. Illumination Settings: Illumination intensity is set as 8000lx.

2.2.1 The method of measuring *chlorella* solute concentration

Adopt Hemocytometer Measurement to determine concentration of *chlorella* solute [7], and use optical microscope to make quantitative analysis for number of *chlorella* in unit-volume water, and during the process of measurement, implement multiple measurements and take the average value as the final result. The specification of haematimeter board is 16×25.

2.2.2 The method of measuring dissolved oxygen of *chlorella* solute

Adopt iodometric method to measure dissolved oxygen [8], and implement multiple measurements and take the average value as the final result. Respectively add 1 ml MnSO4 solute and KI—NaOH solute into an iodine flask of *chlorella* solute with *V1* volume, then put the cap back on the iodine flask (airless), so that dissolved oxygen in the solute is quantitatively translated into brown deposit of manganic compound. Then add 1 ml H_2SO_4 to dissolve the deposit, and then pour all the solute into iodine flask.

When $Na_2S_2O_3$ becomes light yellow, add 1 ml faecula till $Na_2S_2O_3$ becomes achromatous, and the solution volume is up to V thereinto.

The formulas to calculate the dissolved oxygen content in *chlorella* solute:

$$DO(mg/L) = (8.000 \times 1000CV)/V_1 \qquad (1)$$

Where, C indicates concentration of $Na_2S_2O_3$ (mol/L).

2.2.3 The method of measuring chlorophyll of *chlorella*

Adopt *The Specification for Marine Monitoring* (GB 17378.7-2007) to measure chlorophyll. Make *chlorella* and 10g/L MgCO3 solution well mixed, and pump filtration by microfiltration membrane. Then add 10 ml 95% acetone solution and shake up and extract 24h in dark and in refrigerator. Centrifugate *chlorella* 10min by high-speed freezing centrifuge with 4000 r/min at 4℃. Measure the absorbance value at 750、664、647 and 630 nm, and use the absorbance value at 750 nm to calibrate others. Then take the calibrated absorbance value E_{664}, E_{647} and E_{630} in nether formulas.

$$\rho_{chl-a} = (11.85E_{664} - 1.54E_{647} - 0.08E_{630}) \times \frac{v}{V \cdot L} \qquad (2)$$

Where, ρ_{chl-a} indicates content of chlorophyll (μg/L), v indicates volume of extracted liquid (L), L indicates optical path of colorimetric ware (cm).

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3 Results

3.1 The influence temperature takes to growth of *chlorella*

Process *chlorella* solute at different temperatures and measure its concentration every an interval (2.5 hours), then calculate its average increment. See the following table for details.

Tab.1 Content of *chlorella* at different temperature (illumination: 8000lx)

NO.	Processing Condition	Concentration of *Chlorella* Before Being Processed (10^4/ml)	Concentration of *Chlorella* After Being Processed (10^4/ml)			Average Increase Rate (10^4/ml)
			2.5h	5.0h	7.5h	
1	5℃	309.222	311.348	316.670	318.000	2.926
2	10℃	333.551	335.766	337.588	339.333	1.927
3	15℃	303.404	309.432	310.211	314.326	3.641
4	20℃	280.645	291.049	296.370	306.447	8.601
5	25℃	310.450	320.343	339.433	346.343	11.964
6	30℃	320.430	324.119	327.000	330.454	3.341
7	35℃	286.332	287.334	287.40	286.431	0.109

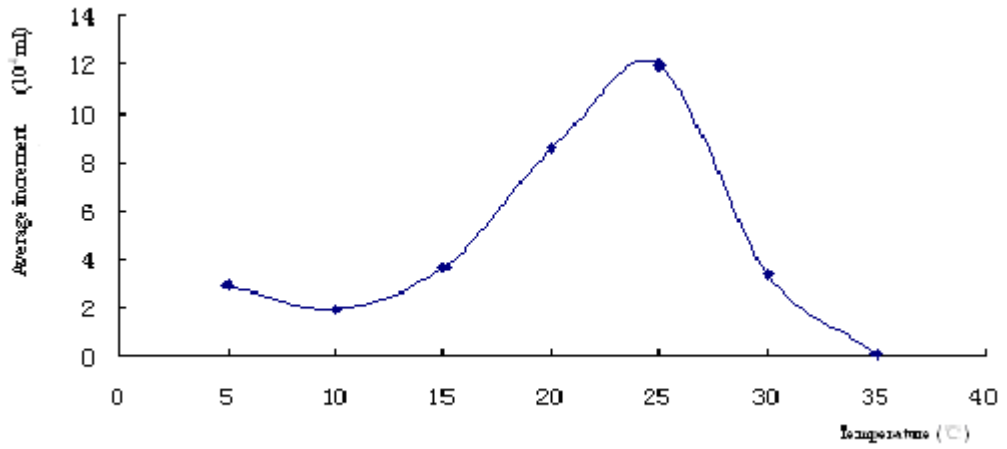

Fig. 1 Growth of *chlorella* at different temperature

According to the analysis in the increment of *chlorella* in Figure 1, the concentration of *chlorella* with a temperature scope of 5℃~35℃ gets different increase. Reproduction increment of *chlorella* is up to 8.601~11.964×10^4/ml with a temperature scope of 20~25℃. Fast growth speed indicates that the temperature in this scope is suitable for growth of *chlorella*. Reversely, the reproduction increment of *chlorella* is within the scope of 0.109 ~ 3.641×10^4/ml when temperature is amid 5~15℃ and 30~35℃. Small increment means growth of *chlorella* gets restrained and reproduction speed is impacted when temperature is within the aforesaid two scopes.

3.2 The influence temperature takes to dissolved oxygen in *chlorella* solute

With invariable illumination intensity (8000lx) and within the temperature scope of 5~30℃, the change of dissolved oxygen in *chlorella* solute is illustrated in the table 2.

Tab.2 Dissolved oxygen content at different temperature (illumination: 8000lx)

NO.	Processing Condition	Concentration of Dissolved Oxygen Before Being Processed (mg/l)	Concentration of Dissolved Oxygen After Being Processed(mg/l)			Concentration Difference of Dissolved Oxygen(mg/l)
			2.5h	5.0h	7.5h	
1	5℃	9.139	10.156	10.244	10.245	0.369
2	10℃	7.745	7.143	8.668	8.885	0.380
3	15℃	8.442	9.306	11.008	12.066	1.208
4	20℃	8.004	15.466	19.556	22.656	4.884
5	25℃	9.524	16.015	20.444	25.007	5.161
6	30℃	8.991	14.228	17.447	21.008	4.005
7	35℃	9.116	9.114	9.106	9.221	0.035

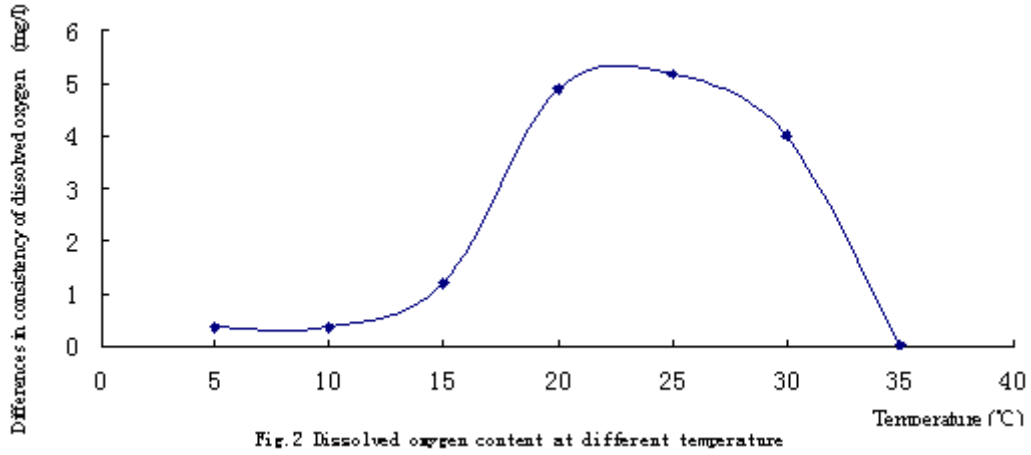

Fig.2 Dissolved oxygen content at different temperature

Figure 2 shows that the increasing rate of dissolved oxygen in the *chlorella* solute is distinctly different with a temperature scope of 5~35℃.It has a high level concentration difference of dissolved oxygen about 4.005~5.161 mg/l with a temperature scope of 20~30℃, and the maximum increment of dissolved oxygen is up to 5.161mg/l at 25℃. It has a lower level concentration difference of dissolved oxygen with a temperature scope of 5~15℃ and at 35℃ than the difference with a temperature scope of 20~30℃.

3.3 The influence temperature takes to content of chlorophyll in *chlorella* solute

With invariable illumination intensity (8000lx) and within the temperature scope of 5~30℃, the change of content of chlorophyll in *chlorella* solute is illustrated in the table 3.

Tab.3 chlorophyll content at different temperature (illumination: 8000lx)

NO.	Processing Condition	Content of chlorophyll Before Being Processed(μg/L)	Content of chlorophyll After Being Processed(μg/L)			Content Difference of chlorophyll(μg/L)
			2.5h	5.0h	7.5h	
1	5℃	0.731	0.754	0.785	0.782	0.017
2	10℃	0.885	0.914	0.925	0.956	0.024
3	15℃	0.724	0.741	0.775	0.813	0.030
4	20℃	0.772	0.852	0.953	0.994	0.074
5	25℃	0.801	0.913	0.984	1.042	0.080
6	30℃	0.812	0.864	0.932	0.981	0.056
7	35℃	0.727	0.756	0.759	0.755	0.009

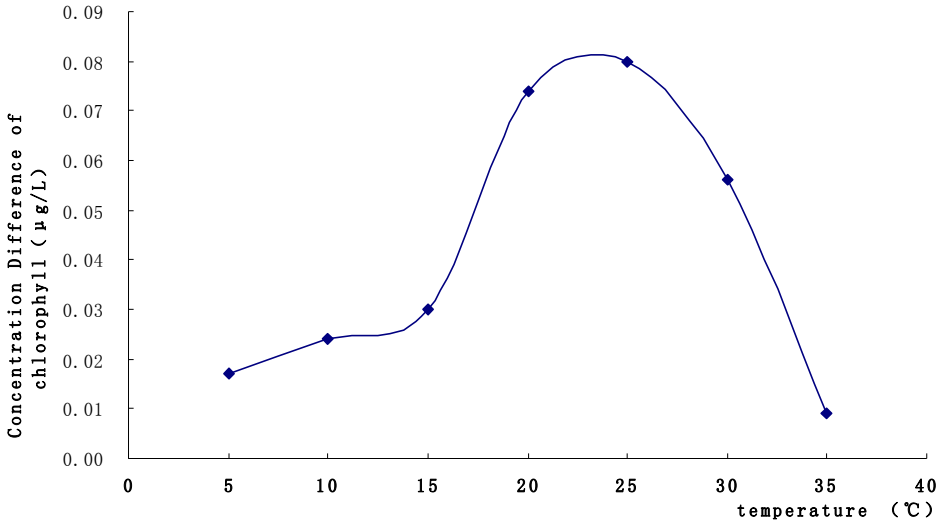

Fig.3 chlorophyll a content at different temperature

It is found that the content difference of chlorophyll in the *chlorella* solute is proportion to the temperature when temperature is within the scope of 5~25℃(figure 3), and the maximum increment of content of chlorophyll is up to 0.080μg/l at 25℃; on the contrary, the increment of content of chlorophyll is reverse proportion to temperature amid 25~35℃, and the maximum value decreases to 0.009μg/l from 0.080μg/l.

4 Conclusion

The experimental results indicate that biological quantity of *chlorella* gets different increase when temperature is amid 10~35℃. The reproduction increment of *chlorella* is within the scope of 0.109 ~ 3.641×10^4/ml when temperature is amid 5~15℃ and 30~35℃. Small increment means growth of *chlorella* gets restrained and reproduction speed is impacted when temperature is within the aforesaid two temperature scopes; reversely, reproduction increment of *chlorella* is up to $8.601\sim11.964\times10^4$/ml with a temperature scope of 20~25℃. Fast growth speed indicates that the temperature in this scope is suitable for growth of *chlorella*. Therefore, in the light of the above-mentioned data analysis, we can draw a conclusion that the most appropriate temperature for incubation of *chlorella* is 25℃.

The increasing rate of dissolved oxygen in the *chlorella* solute is proportion to the temperature when temperature is within the scope of 5~25℃, and furthermore, the maximum increment of dissolved oxygen is up to 5.161mg/l at 25℃; on the contrary, the increment of dissolved oxygen is reverse proportion to temperature amid 25~35℃, and the maximum value decreases to 0.035mg/l from 5.161mg/l. Based on the aforesaid data analysis, we draw a conclusion that the change trend of dissolved oxygen in the

chlorella solute is nearly the same as that of the growth rate of *chlorella*.

The content difference of chlorophyll in the *chlorella* solute is proportion to the temperature when temperature is within the scope of 5~25℃, and the maximum increment of content of chlorophyll is up to 0.080μg/l at 25℃; on the contrary, the increment of content of chlorophyll is reverse proportion to temperature amid 25~35℃, and the maximum value decreases to 0.009μg/l from 0.080μg/l. Accordingly, we draw a conclusion that content difference of chlorophyll in the *chlorella* solute is nearly the same as that of the growth rate of *chlorella*.

According to the above analysis, we are sure it is at 25℃ that *chlorella* gets the fastest growth and reproduction, and the maximum dissolved oxygen and content chlorophyll in *chlorella* solute.

References

[1] Chen Ying, Li Wenbing, Sun Yongru: The current status and prospect on the research and application of chlorella's biological technology, *J. Biological Engineering Development*, **6**(1998), 12-16.

[2] Li Yonghan: Aquatic Feeding Attractant Biology, Dalian, Dalian Press,2000.

[3] Wang Zhen, Wang Changhai: The Research on Incubation Conditions of, *J. The Journal of Yantai University (Natural Science and Engineering Edition),* **6**(2005), 206-211.

[4] Chen Mingyao: Biological Feeding Attractant Incubation, Beijing, Agricultural Press of China, (2002), 27- 65.

[5] Wang Ping, Zhang Qianqian, Wang Xiuling: The Influence Temperature and Illumination take to Growth of Two Red-Tide Diatom, *J.Marine Environmental Science*, **1**(2006), 38-40.

[6] Lin Xia, Lu Kaihong, Qian Yun Xia: How A Couple Of Ecological Factors Impact The Growth of Phaeodactylum tricornutum Bohlin, *J. The Journal of Ningbo University*, **1**(2003), 44-48.

[7] Cai Jun: The Research of Breaking the Waste Beer Yeast Cellswall, *J. Liquor Making*, **4**(2001), 104-105.

[8] Chen Hao, Su Hang, Li Qing: The Measurement of Dissolved Oxygen in the Water, *J.The Journal of Analytical Science* **2**(2005), 215-216.

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